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Genotoxicity of Gamma Irradiation in
L5178Y Mouse Lymphoma Cells

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16. ABSTRACT The L5178Y mouse lymphoma assay has been widely used in short-term mutagenicity testing. Research into the types of genetic damage detected at the thymidine kinase locus indicates that the assay may be capable of evaluating not only the potential gene mutagenicity but also the clastogenicity of a test chemical. The ability to distinguish between the two types of genetic damage is based upon the observation that the mutants can be separated by colony size, using an automatic colony counter, into σ , small colonies and λ , large colonies. In addition σ mutants appear to represent chromosomal alterations to that chromosome 11 carrying the TK locus, while λ mutants may represent smaller-scale, single-gene mutations. The process of evaluating this hypothesis includes the testing of agents of known clastogenicity and an evaluation of the relative proportions of the σ and λ mutant frequencies. Several compounds known to be clastogens have already been shown to induce a significant proportion of σ mutants. These include methyl methanesulfonate, benzo(a)pyrene, 2-acetyl aminofluorene, N-methyl-N'-nitro-N-nitrosoguanidine, and dimethylnitrosamine. A study of classic clastogenic agents would be incomplete without evaluating either X-ray or gamma irradiation. We report here the results of experiments analyzing gross aberrations, sister chromatid exchange, and thymidine kinase gene mutation (including σ and λ mutant frequency) following exposure of TK ⁺ /3.7.2C mouse lymphoma cells to gamma irradiation.			
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Genotoxicity of γ -irradiation in L5178Y mouse lymphoma cells

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Summary

The ability of γ -irradiation to induce gene mutation at the thymidine kinase locus and gross chromosome aberrations in L5178Y TK⁺/3.7.2C mouse lymphoma cells was evaluated. Positive results were obtained for both end-points. The majority of mutants were found to be small-colony mutants which correlated with the induction of gross chromosome aberrations.

The L5178Y mouse lymphoma assay, which quantitates gene mutation at the thymidine kinase locus, has been widely used in short-term mutagenicity testing (Jacobson et al., 1978, 1981; Amacher et al., 1979, 1980; Clive et al., 1979; MacGregor et al., 1979; Amacher and Paillet, 1980; Gold et al., 1980; Krell and Jacobson, 1980; Jotz and Mitchell, 1981; Meltz and MacGregor, 1981; Kirby, 1984; Oberly et al., 1984). Research into the types of genetic damage detected at the thymidine kinase locus indicates that the assay may be capable of evaluating not only the potential

gene mutagenicity but also the clastogenicity of a test chemical (Hozier et al., 1981, 1982, 1983, 1985; Moore et al., 1985b,c).

The ability to distinguish between the two types of genetic damage is based upon the observation that the mutants can be separated by colony size, using an automatic colony counter, into σ , small colonies and λ , large colonies (Moore et al., 1985c). In addition σ mutants appear to represent chromosomal alterations to that chromosome 11 carrying the TK locus, while λ mutants may represent smaller-scale, single-gene mutations (Hozier et al., 1981, 1982, 1983, 1985). The process of evaluating this hypothesis includes the testing of agents of known clastogenicity and an evaluation of the relative proportions of the σ and λ mutant frequencies. Several compounds known to be clastogens have already been shown to induce a significant proportion of σ mutants as well as λ mutants (Moore et al., 1985c). These include methyl methanesulfonate, benzo[a]pyrene, 2-acetylaminofluorene, N-methyl-N'-nitro-N-

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nitrosoguanidine, and dimethylnitrosamine. We are in the process of evaluating several of these compounds for their clastogenicity to mouse lymphoma cells (Doerr and Moore, unpublished data). A dye, C.I. Solvent Yellow No. 33, has been shown to be clastogenic to mouse lymphoma cells and produces a significant proportion of small colonies (Moore et al., 1985a), as well as large-colony mutants.

A study of classic clastogenic agents would be incomplete without evaluating either X-ray- or γ -irradiation. We report here the results of experiments analyzing gross aberrations and thymidine kinase gene mutation (including σ and λ mutant frequency) following exposure of TK^{+/+}-3.7.2C mouse lymphoma cells to γ -irradiation.

Materials and methods

Cells

The TK^{+/+}-3.7.2C heterozygote of L5178Y mouse lymphoma cells (Fischer, 1958) was used. TK^{-/-}-3.7.2C is a subclone of the TK^{+/+}-3.7.2, which, in turn, was isolated as a spontaneous THMG-resistant revertant from the EMS-induced TK-deficient clonal line TK^{-/-}-3.7 (Clive, 1973; Clive and Voytek, 1977).

Cells were maintained in culture according to the procedures of Clive and Spector (1975) with modifications (Clive et al., 1979; Moore and Clive, 1982). Fischer's powdered or liquid Medium for Leukemic Cells of Mice (Gibco) was used. Each lot of horse serum was checked for a clear appearance and ability to support normal growth (cell density $\geq 0.2 \times 10^6$ cells/ml in 4 days) from low cell inocula (200 cells/ml) before purchase from Gibco.

Chemicals

TFT was obtained from Sigma. The agar used for cell cloning was obtained from Baltimore Biological Laboratory.

γ -Radiation exposure

γ -Ray exposures were performed using a ¹³⁷Cesium dual source irradiation (J.L. Shepard,

model 431). The instrument provided an unattenuated dose rate of 21 Gy/min (2100 rad/min). During each exposure, an X-ray monitor (MDH Industries, series 2025) was employed to measure cumulative delivered doses. The resolution of this instrument is 1 mrad/min. Cell suspensions (0.6×10^6 cells/ml in growth medium containing 10% horse serum) in plastic centrifuge tubes were placed on a 12-rpm turntable with the monitor probe centered over them during periods of exposure. Low doses were delivered as necessary through lead attenuators.

Cell culture and mutant selection

Following irradiation, cells were centrifuged and washed twice with fresh medium. Cells for each dosed culture were resuspended in 20 ml of fresh growth medium and placed on a roller drum in a 37°C incubator. Cells were maintained in log phase growth for 2 days expression and then cloned with TFT selection (1 μ g/ml) and without selection (for viable count determination) in BBL agar supplemented cloning medium (Meyer et al., 1985).

Colony counting and sizing

After 9-11 days incubation at 37°C, colonies were counted on an Artek model 880 automatic colony counter using settings that optimize colony

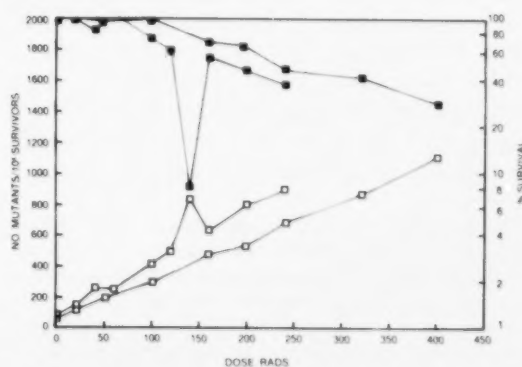


Fig. 1. Dose-response curves for the induction of mutants of TFT⁺ TK^{+/+} 3.7.2C mouse lymphoma cells exposed to gamma irradiation. Results from two separate experiments. Survival calculated according to method of Clive and Spector (1975).

counts, compared to hand counts, without producing a spurious background. These counters have size discriminators that quantitate the colony-size distribution.

Colony-sizing curves were generated by calculating the difference between adjacent counts of up to 16 uniformly spaced settings on the size discriminator. Each of these differences was converted to a mutant frequency by correcting for the percent of the cells plated. The difference in mutant frequency between adjacent size setting was plotted as a histogram.

Cytogenetic analysis

Following the cell irradiation and cell wash, 10 μ M BrdUrd was added to cultures used for

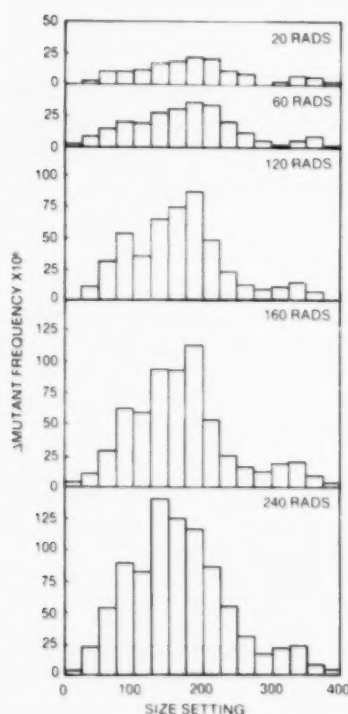


Fig. 2. Size distribution curves for TFT⁺ mutants following exposure of TK^{+/-} 3.7.2C mouse lymphoma cells to γ -irradiation. Colony size increases as the size setting increases. The σ colony peak is shown to the left of size setting 275 in each graph and the λ colony peak to the right of size setting 275.

cytogenetic analysis. Cells were incubated for 9–10 h. Colcemid was added during the last 2 h of incubation. Cells were harvested using a hypotonic KCl solution and fixed in acetic acid:methanol (1:4). Slides were made and cells stained with a fluorescence plus Giemsa technique. Well-spread metaphases showing a near normal number of chromosomes were scored. Only first-division metaphases were analyzed for aberrations, which consisted of chromatid and chromosome breakage, as well as rearrangements. Gaps were recorded, although not included in the analysis. All slides were blind-coded prior to scoring.

Results and discussion

γ -Irradiation was found to be mutagenic to the TK^{+/-} 3.7.2C mouse lymphoma cells. Fig. 1 shows the dose-response curves for two separate experiments. The results were quite reproducible for the two experiments. An analysis of the relative

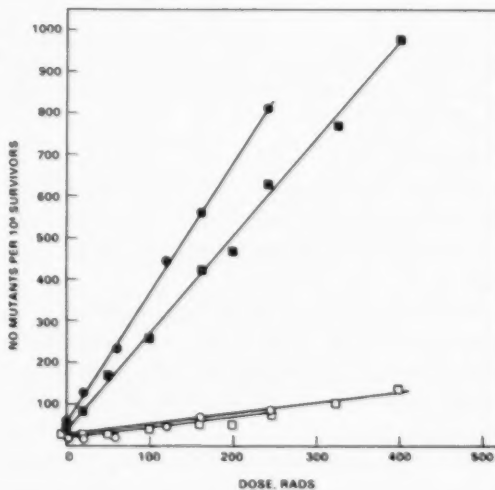


Fig. 3. Dose-response curves for the induction of TFT⁺ mutants following γ -irradiation. For this graph the total mutant frequency has been divided into the σ mutant frequency (closed symbols) and the λ mutant frequency (open symbols). The total mutant frequency from the two separate experiments is shown in Fig. 1. Sizing curves were not generated at all doses; thus, not all dose points could be separated into σ and λ mutant frequencies.

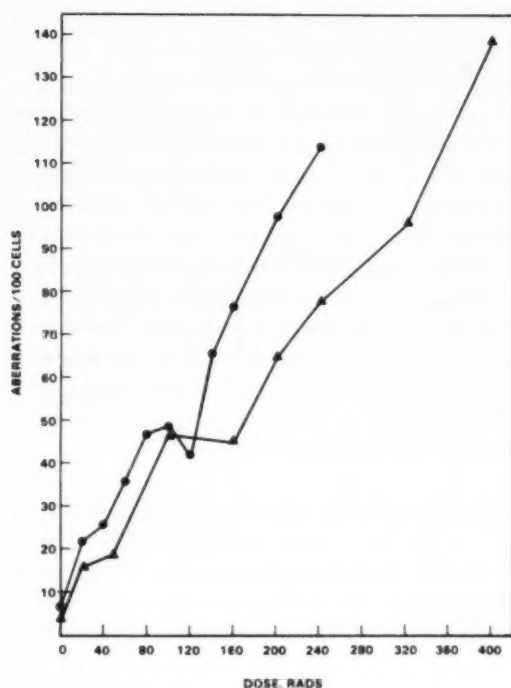


Fig. 4. Dose-response curves for chromosomal aberration induction in TK^{-/-} 3.7.2C mouse lymphoma cells exposed to γ -irradiation. Results from two separate experiments. The mutagenicity data for these experiments are shown in Fig. 1.

proportion of σ and λ TFT-resistant (TFT^r) mutants revealed a predominance of σ mutants that increased dramatically with increasing dose (Figs. 2, 3).

As would be predicted by the large proportion of σ TFT^r mutants, the γ -irradiation induced a significant increase in the number of gross aberrations per 100 cells (Fig. 4). A positive response was observed at doses as low as 20 rad. The doses used for the cytogenetic analysis were those analyzed for gene mutation at the thymidine kinase locus, and resulted in reasonable levels of cytotoxicity as measured by the relative total growth, which in all cases was greater than 20% (Fig. 1).

γ -Irradiation and X-irradiation have been widely studied in mammalian test systems where they have been separately tested for the ability to induce gene mutation or chromosome aberrations. Many

of these dose-response studies of induced mutation have employed rodent cells as the test system (Kao and Puck, 1969; Carver et al., 1976; O'Neill et al., 1977; Thacker et al., 1977; Nakamura et al., 1982; Nishi et al., 1984). γ -Irradiation has also been analyzed for its ability to induce chromosome aberrations in in vitro mammalian test systems (Purrott and Lloyd, 1972; Carrano, 1973a,b; Lloyd et al., 1975; Roberts and Holt, 1982; Lloyd et al., 1984).

The experiments reported here correlate the σ TFT^r mutant frequency in mouse lymphoma cells (Fig. 3) with the ability to induce chromosome aberrations in the same cells (Fig. 4). These results are consistent with the hypothesis that σ mutants predict clastogenicity and argue for the routine determination of σ and λ mutant frequencies.

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